University of California at Berkeley Department of Nutritional Sciences

Semi-Annual Progress Report

Jan. 1, 1967 - June 30, 1967

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Title: Nutritional Requirements and Breeding Behavior of Perognathus

1. Nutritional Requirements:

A semi-synthetic diet has been developed which appears to be adequate to maintain weight, good health and normal behavior in P. penicillatus, P. intermedius and P. longimembris. It is a pelleted diet containing soy protein, cornstarch, corn oil, fiber, vitamins and minerals (Table 1). The composition of the mineral supplement turned out to be of crucial importance to the success or failure of the diet. It contains a high ratio of potassium to sodium and no inorganic phosphorus but relatively large amounts of magnesium and calcium-glycerphosphate. The reason for the success of this type of mineral supplement for the pocket mouse is unknown. The sunflower meal on which they seem to thrive indefinitely also contains a high potassium to sodium ratio and close to 2% phosphorus (expressed as P_2O_5) (Table 2). However, the ability of these animals to utilize phosphorus in the forms present in the seeds compared to inorganic phosphates or to glycerophosphate is unknown. Consequently the proportions of Ca:Mg which is actually absorbed and utilized is also unknown. It seems clear, however, that the importance of a proper mineral composition of the diet is related to the critical water economy of these animals. This is indicated by experiments showing that the animals will do well on an otherwise lethal diet when it is supplemented with fresh carrots. (See Progress Report Jan.-Feb. 1967)

Portions of these results have been presented in a paper at the Pacific Slope Biochemical Conference, June 1967 (Development of a semisynthetic diet for the Pocket Mouse, Perognathus; Gene A. Spiller and R. Ostwald). A paper to be submitted to J. Nutr. is in preparation. We are now in the process of a) testing this diet for its ability to support growth, lactation and reproduction and b) using the diet to investigate a number of problems concerning the requirements and utilization of selected nutrients (for outline see Application for continued support July 1, 1967 - June 30, 1968)

2. Induction of Breeding Condition:

a. Administration of hormones

We have found that a diet containing estrogens induces an estrus which, however, turned out to be infertile. This treatment even when supplemented with a progestational agent appears not to induce the full endogenous hormonal response necessary for ovulation and/or implantation.

Injection of the gonadotropin Pregnant Mare Serum (PMS) has produced limited but encouraging success. Of ten female P. penicillatus given a single dose of 3 IU PMS, eight came into estrus from 48 hours to 6 days later, but only one produced a litter. Higher doses of PMS with and without Human Chorionic Gonadotropin (HCG) are now being tried, and initial results indicate that a single dose of 6 IU PMS produces estrus from 24 to 72 hours later. Females given such treatment have been mated; it is too early to say if they are pregnant.

b. Manipulation of the environment

(1) Physical variables. Groups of P. pen. have been exposed in the "Climatron" (an environmental chamber permitting a wide range of temperature and humidity conditions) to those aspects of a "winter-to-spring" cycle which might be expected to influence the induction of breeding condition. The light, temperature and humidity schedule (Table 3) was designed to approximate the conditions found inside burrows of pocket mice in southern Arizona. The results of this experiment show that the "Climatron" operates very well indeed, that the mice go into hibernation during the simulated winter, and that both male and female animals became sexually active by the 8.-10. week of "Spring". Of 9 attempted matings, 3 females became pregnant: two delivered litters of 5 and 6 pups each and one aborted a few days before birth was expected; three did not become pregnant; and it is too early to say for the last 3. Because of the coincidence of "late spring" in the Climatron with April-May (Table 3) we will repeat this experiment on a schedule which will produce spring in Sept.-Oct. at which time the mice are known not to breed in their natural habitat.

(2) Social variables. Female P. penicillatus can be brought into estrus when kept in cages which allow for visual and olfactory contact with a male by means of screen dividers. Of 24 females so treated 17 came into estrus within 12 days after being placed into the cage, four came into estrus between 12 and 26 days, and three did not respond. Eight successful pregnancies resulted from 15 attempted matings. This experiment will also have to be repeated at a different time of year to ascertain whether the proximity of a male alone is sufficient stimulus to initiate the whole sequence of hormonal events or whether it represents a trigger mechanism for only the last steps involved in the sequence.

There appear to be major differences between subspecies in the ease with which breeding can be induced. In contrast to the success with P. pen. by the methods outlined under 2a and 2b(2) above, neither of them produced estrus in P. longimembris.

We are now investigating the effects of a combination of hormone treatments and screen-divided cages on this subspecies.

A paper describing the successful breeding of P. pen. to be submitted to J. Mamm. is in preparation.

c. Dietary factors

We have tried a number of dietary supplements for their ability to bring the mice into breeding condition. In addition to vitamin supplements, cactus sprouts, meal worms, lettuce and carrots which we have described previously (semi-annual report Jan. 1966-June 1966), we have now tried wheat germ oil, yeast, dessicated liver and the provision of a salt-lick. The composition of this diet is given in Table 4. The diet was supplemented with fresh carrots twice a week and the animals had access to a crystal of rocksalt glued to the side of the cage. Laboratory lighting was on a daily cycle corresponding to the natural day length for the period of the experiment (Jan. 16 to March 18.). No sexual activity in either sex was observed during the two months. It therefore appears that we either have not found the correct nutrients or that other conditions more important for the induction of breeding condition have not been met, and that this lack overshadowed the contribution of dietary variables (see 2b-1 and 2b-2 above).

Plans for the Immediate Future

In addition to the studies outlined above we are in the process of studying the effects of different diets on certain physiological parameters of the mice such as body composition, electrolyte balance, organ histology and hematology. The results of these studies should provide clues for the reasons for the failure or success of the diets employed.

In view of the importance of the mineral composition of the diet we are also planning to study the absorption and excretion of Ca, Mg and P.

Summary

1) A semi-synthetic diet adequate for long-term maintenance of adult P. penicillatus, longimembris, and intermedius has been developed. The presence of a high ratio of K/Na, Mg/Ca and the substitution of inorganic phosphates by glycerophosphate turned out to be the crucial factors for success.
2) Female P. pen. can be brought into breeding condition by three methods:
   a) Treatment with Pregnant Mare Serum.
   b) Exposure to a sequence of "winter" and "spring" conditions in an environmental chamber.
   c) Visual and olfactory contact with males in screen-divided cages.

3) Eleven litters comprising approximately 50 pups have been produced: 1 litter by method a, 2 by method b, and 8 by method c. In addition to these 2 litters were obtained from mice who had come spontaneously into estrus during the report period.
4) Future plans involve a) elucidation of the reasons for the exotic mineral requirements of Per.; b) attempts to repeat the breeding studies during periods of the calendar year when the mice are known not to breed spontaneously; c) attempts to use the breeding methods for other subspecies of Per.

Table 1

Composition of Semi-Synthetic Diet

	Per 100 g diet (g)
Soy protein	22
Corn starch	58
Corn oil	9
Fiber (Solka Flock)	7
Gum Arabic, USP	1
Mineral Mix:	
NaCl	0.2
KCl	0.25
K citrate	1.0
Mg glycerophosphate	1.5
$MgSO_4$	0.25
Ca glycerophosphate	0.5
Fe citrate	0.25
Traces of iodine, copper, zinc and manganese	
	(mg)
Vitamin Mix:	
Choline	675
Riboflavin	2.0
Thiamine	1.5
Niacin	1.5
Pyridoxine	0.5
Folic acid	12.5
Biotin	0.05
Inositol	0.5
PABA	6.0
Menadione	50.0
Tocopherol	30.0
B_{12}	2.5 µg
A	250 IU
D	100 IU

Table 2

Composition of Sunflower Meal - Millet Diet	Protein	18 %
	Fat	25 %
	Fiber	5-6 %
	Moisture	10 %
	CHO	50 % approx.
Mineral Composition of Sunflower Meal Ash: (ash content of meal is 4.7%)	Na	0.22 %
	K	17.20 %
	Ca	2.82 %
	Mg	10.10 %
	Fe	0.10 %
Sunflower Meal Content of Anions:	Cl	0.11 %
	SO_4	0.25 %
	P_2O_5	1.92 %

Table 3

Temperature, Humidity and Light Schedule of "Climatron"

"Seasons"	Week	Date	Temperature	Humidity 50% constant	Lights On	Lights Off	Day length hr
Acclimation	1	2/7	Lowered to 50°F		8:0	16:0	8
"Winter"	2	2/14	Constant 50°F		"	"	
	3				"	"	
	4				"	"	
	5				"	17:00	9
"Early Spring"	6	3/14	Gradually raised		"	17:30	9 1/2
					"	18:00	10
					"	18"30	10 1/2
	7				"	19:00	11
"Spring"	8	3/28	Cycling between 66 and 75°F (24-hr cycle)		"	19:30	11 1/2
					"	20:00	12
	9				"	20:30	12 1/2
					"	21:00	13
	10				"	21:30	13 1/2
	11				"	22:00	14
					"	22:30	14 1/2
	12				"	23:00	15
					"	23:30	15 1/2
	13				"	24:00	16
	through 20	6/19			Continued at these settings through week 20.		

Table 4

Composition of Diet Used in an Attempt to Induce Breeding by Dietary Means

	%	grams
Sunflower meal	46	450 (1 lb.)
Millet meal	46	450 (1 lb.)
Wheat Germ Oil	1.0	10
$MgSO_4$	0.2	2
Torulla Yeast	3.0	30
Desicated liver	3.0	30